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CRANE 1623
SN 09/032,972 03/02/98
Krotz et al.

Methods for Synthesis of Oligonucleotides

• Before the Board of Appeals

John A. Harrelson
For Appellant

MAILED
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GROUP 2900

Examiner's Answer

This is in response to appellant's brief on appeal filed July 25, 2003.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims.

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final.

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention.

The summary of invention contained in the brief is correct.

(6) Issues.

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims.

The rejection of claims **1-42** stand or fall together because appellant's brief does not include a statement that this grouping of claims does not stand or fall together. See 37 C.F.R. §1.192(c)(7).

(8) Claims Appealed.

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record.

The following is a listing of the prior art of record relied upon in the rejection of claims under appeal.

Number	Name	Date
5,705,621	**Ravikumar**	01/06/98
4,973,679	**Caruthers et al.**	11/27/90
5,548,076	**Froehler et al.**	08/20/96
4,458,066	**Caruthers et al.**	07/03/84
4,500,707	**Caruthers et al.**	02/19/85
5,132,418	**Caruthers et al.**	07/21/92

Sproat et al. (I), "2'-O-Methyloligoribonucleotides: Synthesis and Applications," Ch. 3 in Oligonucleotides and Analogues - A Practical Approach, Eckstein (ed.), IRL Press, New York, NY, 1991, only title and text pages 49-86 supplied, see especially p. 52.

Conway et al., "Site-Specific Attachment of Labels to the DNA Backbone," Ch. 9 in Oligonucleotides and Analogues - A Practical Approach, Eckstein (ed.), IRL Press, New York, NY, 1991, only title and text pages 211-239 supplied, see especially p. 218.

Atkinson et al., "Solid-Phase Synthesis of Oligonucleotides by the Phosphite Triester Method," Ch. 3 in Oligonucleotide Synthesis - A Practical Approach, Gait (ed.), IRL Press, Washington, DC, July, 1985, only title and text pages 35-81 supplied, see especially p. 80.

Sproat et al. (II), "Solid-Phase Synthesis of Oligodeoxynucleotides by the Phosphotriester Method," Ch. 4 in Oligonucleotide Synthesis - A Practical Approach, Gait (ed.), IRL Press, Washington, DC, July, 1985, only title and text pages 83-115 supplied , see especially p. 111.

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims.

First Obviousness Rejection

The following is a quotation of 35 U.S.C. §103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> "A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made."

Claims **1-42** are rejected under 35 U.S.C. §103(a) as being unpatentable over **Ravikumar '621** (PTO-892 ref. **A**) in view of

Caruthers et al. '679 (PTO-892 ref. **G**) and further in view of **Froehler et al. '076** (PTO-892 ref. **H**) and further in view of **Sproat et al.(I)** (PTO-892 ref. **W**), **Conway et al.** (PTO-892 ref. **Y**), **Atkinson et al.** (PTO-892 ref. **Z**), and **Sproat et al.(II)** (PTO-892 ref. **RA**).

The instant claims are directed to entirely conventional, 7 step oligonucleotide syntheses conducted using an automated device to execute steps 2-6 {aka steps b) through f)}, wherein the two variations from the prior art are i) the choice of solvent or solvent mixture present for deprotection step (c) and ii) the choice of substrate as a linear oligonucleotide as opposed to the branched oligonucleotide of the prior art.

Ravikumar '621 (PTO-892 ref. **A**) discloses entirely conventional oligonucleotide synthesis wherein the solvent for the coupling step is acetonitrile in the examples and the P-protecting group varies from the conventional phosphorus-ester protecting group. At column 3 this reference refers to several different patents which disclose the solid phase synthesis of oligonucleotides including three Caruthers et al. patents now cited herein as PTO-892 references **I, J and K**. Each of these Caruthers et al. patents discloses the automation of the synthesis of oligonucleotides via process steps closely analogous to, if not identical with, the process steps claimed herein, the most detailed disclosure occurring in Caruthers et al. '418 (PTO-892 ref. **K**). In the **Ravikumar '621** patent at column 10, lines 1-16, a generic disclosure of the process steps leading to an oligonucleotide is presented, including acid-mediated deprotection of the 5'-hydroxyl moiety of a solid-support-attached nucleoside. However, no disclosure of any preferred solvent for the required acid reagent is included. In the same column at line 50, the removal of 5'-hydroxyl protection by contact with acid from a solid-support-attached oligonucleotide is also taught without specifying any

particular solvent. At column 14, lines 5-28, a more complete disclosure of possible 5'-hydroxyl protecting groups is provides along with a list of acids effect to deprotect, but no preferred solvents are listed. At column 18, lines 37-41, deprotection is accomplished by contact with a solution of dichloroacetic acid in dichloromethane, conditions repeated in subsequent experimental procedures. The choice of any particular deprotection solvent is therefore apparently a choice within the purview of the ordinary practitioner in view of this disclosure. This reference does not disclose the particular mixture of solvents selected for use in the instant claimed processes.

Caruthers et al. '679 (PTO-892 ref. **G**) at column 5, lines 10-14, teaches the use of "... any solvent which will dissolve the reactants ..." including a list of specific organic solvents for phosphoramidite-intermediate-based oligonucleotide synthesis. The context of this statement suggests that Caruthers was making reference to the coupling step. However, the same generic teaching appears to also apply to the deprotection step where four different solvent/reagent systems were disclosed by Caruthers as effective in the 5'-O-detritylation process:
(1) see column 16, Table IV, footnote 1 ($ZnBr_2$ in nitromethane);
(2) see column 16, Table V, footnote 1 (toluenesulfonic acid in chloroform:methanol (7:3));
(3) see column 18, lines 26-28 ($ZnBr_2$ in nitromethane:methanol (19:1)); and
(4) see column 19, lines 47-50 (80% acetic acid).
This reference does not disclose the particular mixture of solvents selected for use in the instant claimed processes.

Froehler et al. '076 (PTO-892 ref. **H**) discloses the use of H-phosphonate intermediates for the coupling step in the synthesis of oligonucleotides and phosphorothioate analogues thereof, including

reference to the automated synthesis thereof using a "Biosearch Model 8600 DNA synthesizer" at column 9, lines 22-23. This reference also teaches the use of " ... an anhydrous organic solvent, preferably pyridine/acetonitrile ...," at column 5, lines 26-28. This "what ever works best" philosophy apparently also applies to the deprotection step; see column 5, lines 38-47. The last line of this portion of column 5 is particularly instructive. After listing 3 (three) different deprotection reagent/solvent mixtures, **Froehler** suggests a very flexible "whatever works" approach by further stating that "[o]ther deprotection procedures suitable for other known protecting groups will be apparent to the ordinary practitioner." This reference does not disclose the particular mixture of solvents selected for use in the instant claimed processes.

Sproat et al. (I) (PTO-892 ref. **W**) discloses at p. 52, (lines 2 and 18) that toluene is useful for the purification of synthetic nucleoside intermediates. Additionally, this reference discloses at pp. 64 (Protocol 17, step 3) and page 70 (Protocol 25, step 4) that benzene is a solvent for key oligonucleotide synthesis reagents and for nucleoside-3'-O-phosphoramidites, and may be used to co-evaporate triethylamine therefrom.

Conway et al. (PTO-892 ref. **Y**) is directed to the chemical synthesis of labeled DNA and at p. 218, Section C, Subsection 2, discloses the specific use of toluene as an effective solvent for dissolution of pyridine-contaminated dinucleoside monophosphorothioate d[Cp(s)C] prior to co-evaporative removal of the pyridine/toluene mixture therefrom. The instant reference does not disclose that toluene is used in the coupling step required to make this compound.

Atkinson et al. (PTO-892 ref. **Z**) discloses at p. 43 in section (xvii), that toluene is useful to dissolve the 3'-O-phosphoramidites of 2'-deoxyadenosine, 2'-deoxycytidine, and 2'-deoxyuridine as the first step in a re-precipitation or recrystallization process. This reference also teaches at p. 76, section 7.5, "Variation in Procedures," although no specific teaching of the substitution of an aromatic solvent from other solvents used in oligonucleotide synthesis is present in this section. In section 8.7 at p. 80, "toluene" is listed as a reagent useful in the preparation of "Deoxyribonucleoside-derivatized supports." This reference at the noted locations does not disclose the particular set of solvents claimed herein as useful in the coupling step of an oligonucleotide synthesis.

Sproat et al.(II) (PTO-892 ref. **RA**).at p. 84, lines 10 and 9 from the end of the page, discloses that the "[p]urity of solvents and reagents is of the utmost importance as far as reliability and reproducibility of the [oligonucleotide synthetic] method are concerned." This reference also discloses at p. 93, section (xv), that a di-protected adenosine derivative may be effectively dissolved in toluene prior to evaporative solvent removal for the purpose of co-evaporating residues of pyridine therefrom (see also p. 96, section (vi) for a similar disclosure). Additionally, at p. 111, section 7.6, the listing of solvents useful in oligonucleotide synthesis includes both benzene and toluene. This reference at the noted locations does not disclose the particular set of solvents claimed herein as useful in the coupling step of an oligonucleotide synthesis.

The teachings of the prior art **Caruthers '679** and **Froehler '076** references motivate the selection of practically any organic solvent or solvent mixtures which will dissolve the reactants and not otherwise

interfere with the intended synthetic transformation. The first three references (**A, G and H**) and the additional **Caruthers et al.** patents cited by **Ravikumar et al. '621** provide descriptions of conventional prior art processes for making oligonucleotides via phosphoramidite or H-phosphonate intermediates, including the 5'-O-deprotection process step and including details of how the process has been automated in reference **H** and by patents cited in reference **A**. The noted portions of the **Caruthers '679** and **Froehler '076** both teach that the choice of a particular solvent or solvent mixture is a variable clearly within the purview of the ordinary practitioner. The **Sproat et al. (I) (W)**, **Conway et al., Atkinson et al.**, and **Sproat et al. (II)(RA)** references are each generally directed to oligonucleotide synthesis thereby providing proper motivation to combine with the primary references. The secondary references provide disclosures that at least two different nucleoside-3'-O-phosphoramidites, at least one dinucleotide derivative, and some other nucleoside derivatives may be effectively dissolved in the aromatic hydrocarbon solvents benzene and/or toluene. These disclosures are deemed to provide factually specific motivations for the ordinary practitioner conducting routine experimentation to substitute toluene, benzene, or their closely related aromatic solvent relatives as substitutes for at least a portion of the solvents typically used during the deprotection step in oligonucleotide synthesis. And lastly, in light of the absence of any unexpected results, the choice of substrate (linear vs. branched oligonucleotide) is deemed to not be a basis for finding patentable distinction over the prior art of record. For these reasons the instant process claims are deemed to be lacking in any patentable distinction in view of the noted prior art.

Therefore, the instant claimed oligonucleotide processes would have been obvious to one of ordinary skill in the art having the above cited

references before him at the time the invention was made.

Second Obviousness Rejection

Claims **1-42** are rejected under 35 U.S.C. §103(**a**) as being unpatentable over **Horn et al.** (PTO-892 ref. **WA**; previously cited as PTO-1449 ref. **CB**) in view of **Horn et al.** (PTO-892 ref. **UA**).

The subject matter of the instant claims is described in the previous rejection.

Horn et al. (WA) at page 6965, first complete paragraph (lines 19-28), discloses the use of dichloroacetic acid in toluene for the trityl deprotection step in the synthesis of branched oligonucleotides. Horn notes in particular that a higher than usual (for single deprotection) concentration of dichloroacetic acid effects rapid de-tritylation when multiple de-tritylations must be conducted simultaneously in the parallel extensions of separate oligonucleotide chains is required for the synthesis of multiply branched oligonucleotide "fork and comb" type probes.

Horn et al. (UA) at page 4844, columns 1-2 (following the header "**Oligonucleotide synthesis**"), discloses further details relevant to the application of a mixture including dichloroacetic acid and toluene/methylenechloride to effect the de-tritylation of linear 5'-tritylated oligonucleotide precursors during the process of oligonucleotide chain extension. See particularly page 4844, column 2 at lines 6-8 and 25-28.

The prior disclosures of standard phosphoramidite-type oligonucleotide syntheses of either branched or linear oligonucleotides wherein the de-tritylation step relies on a mixture comprising

dichloroacetic acid and toluene are deemed to be teachings which individually, or in combination, read on the instant claimed process. For this reasons the instant process claims are deemed to be lacking in any patentable distinction in view of the cited prior art.

Therefore, the instant claimed oligonucleotide processes would have been obvious to one of ordinary skill in the art having the above cited references before him at the time the invention was made.

(11.1) Response to Argument; First Obviousness Rejection.

Appellant argues that there is no motivation to combine the multiple references applied in the instant rejection of the instant claims. Examiner respectfully disagrees. The instant first primary reference (**Ravikumar '621**) defines the solvent for the deprotection step of claim **1** only generically until the example wherein a solvent is named, and thereby provides the necessary motivation for an "open to all possibilities" selection of any particular solvent or solvent mixture by the ordinary practitioner.

In support of this "open to all possibilities" view, **Caruthers et al. '679** teaches that " .. any solvent which will dissolve the reactants ..." when selecting solvents for the coupling reaction. Appellant has argued that the four listed solvents in the description of the **Caruthers et al. '679** in the final rejection are not a proper basis for motivation. Examiner respectfully disagrees. First, appellant as mischaracterized the listed solvent mixtures which are, as specifically noted in the description, mixtures listed by **Caruthers '679** as alternative solvents in the 5'-O-detritylation/deprotection process step. The point of listing these was to show that **Caruthers** was not limited to a single solvent or solvent mixture for said deprotection process step, and that therefore

the ordinary practitioner was motivated to select any other solvent or solvent mixture during routine experimentation to optimize the prior art **Caruthers et al. '679** process.

Appellant at page 6 of the brief argues that **Froehler et al. '076** also fails to provide motivational guidance concerning solvent choice for the deprotection step. Examiner respectfully disagrees and, as noted in the final rejection, concludes that **Froehler** also leaves the door open to the ordinary practitioner to select any available solvent or solvent mixture for the 5'-O-detritylation/deprotection step during the process of routine experimentation to optimize the deprotection step in the prior art process of **Froehler et al. '076**.

Appellant then argues that the secondary references **Sproat (I), Conway, Atkinson, and Sproat (II)** by their showings of solubility of intermediates in oligonucleotide synthesis in toluene and benzene are evidence in support of an obvious to try argument and not a proper source of solvents as motivated by the primary references. Examiner respectfully disagrees, and argues that the ordinary practitioner is invited by the motivational guidance of the above noted primary references to select solvent alternatives from the prior art. By presenting the four secondary references, examiner was providing evidence that "aromatic" solvents were known in the art to be useful in oligonucleotide synthesis, and therefore were available to be selected by the ordinary practitioner during routine experimentation.

And in conclusion, examiner notes again that applicant has as yet not provided any sworn showing in support of unexpected results. Therefore, examiner continues to maintain that appellant's selection of aromatic solvents for the deprotection step in an otherwise conventional synthesis of oligonucleotides is not patentably distinguishable over the

prior art without such a showing.

(11.2) Response to Argument; Second Obviousness Rejection.

Appellant at page 7 of the brief argues initially that the combination of references cited was not properly motivated. Examiner respectfully disagrees. First examiner notes that both the "**UA**" reference and the "**WA**" references are directed to oligonucleotide synthesis, the "**UA**" reference including disclosure of how to make both a linear and a branched oligonucleotide, while the "**WA**" reference is directed to "Fork and Comb" type branched oligonucleotide syntheses. In view of this description and the absence of detailed facts in support of applicant's argument, examiner fails to see the logic of appellant's argument, and also therefore, finds appellant's first argument to be very brief and conclusory.

Appellant then argues that the instant cited art fails because it does not disclose applicant's particular process modification in the synthesis of a "linear" oligonucleotide. Examiner disagrees with this argument because appellant's argument is applying the anticipation standard, not the obviousness standard.

Appellant then quotes instant claim language ("the solvent consists essentially of an aromatic solvent ... or an aromatic ether solvent") and avers that this language "precludes use of CH_2Cl_2 [containing] solvent." Examiner respectfully disagrees on three grounds. First, the term of art "consisting essentially of" does not entirely exclude toluene/methylene chloride mixtures and secondly, the term "solvent" may be defined narrowly to read on a single solvent or broadly to read on mixtures of solvents with the second broad meaning being more likely in the present

context because of the consistent presence of the generic modifying term "aromatic." And lastly, examiner notes that the "**WA**" reference at page 6965, first full paragraph, discloses that dichloroacetic acid/toluene was found to be superior to dichloroacetic acid/methylene chloride for 5'-O-detritylation when applied to branched DNA's, a teaching which clearly suggests that the former mixture would also work well for 5'-O-detritylation of linear DNA's.

At page 8, appellant then mischaracterized the "**WA**" reference by stating that "the standard deprotecting reagent {dichloroacetic acid/methylene chloride} was found to be ineffective for deprotection of the synthesized branched DNA's." The "**WA**" reference states the facts as follows: "[i]n our early attempts to synthesize branched DNA, we found it difficult to deprotect the multiple intramolecular dimethoxytrityl functions with standard [dichloroacetic acid/methylene chloride]." The term "ineffective" used in appellants argument suggests that this reagent/substrate reaction is "impossible" or "inoperative," whereas the term "difficult" from the **WA** text suggests a slower reaction rate, but does not exclude success.

Appellant then argues, assuming the "ineffective/inoperative" standard above, that dichloroacetic acid/toluene would not be as good as dichloroacetic acid/methylene chloride for trityl group deprotection linear oligonucleotides because the latter reagent would not react at all with the branched contaminants and therefore make purification of linear oligonucleotides from branched contaminants more difficult. While this argument may be correct, appellant has failed to provide side-by-side comparison data to support same and therefore this argument is deemed to be speculative at best and incorrect at worst.

In conclusion appellant argues that there is no reference or

combination of references which fairly teaches the instant claimed subject matter. Examiner respectfully disagrees, noting that reference "**UA**" teaches that the dichloroacetic acid/methylene chloride + toluene reagent is effective in deprotection of both linear and branched oligonucleotides. Therefore, it is not surprising that the reagent dichloroacetic acid/toluene is also effective in deprotection of 5'-tritylated branched oligonucleotides as disclosed by reference "**WA**." The extension of the "**WA**" deprotecting conditions to linear oligonucleotides is therefore deemed to have been no more than a well motivated and therefore obvious variation for the ordinary practitioner seeking through routine experimentation to optimize the deprotection/detritylation step of the standard process for making oligonucleotides.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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